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REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Large-scale multiplex analysis of highly polymorphic loci is needed for practical identification of individuals, e.g., in paternity testing and forensic science, organ-transplant donor-recipient matching, genetic disease diagnosis, prognosis, pre-natal screening, and the study of oncogenic mutations. In addition, the cost effectiveness of infectious disease diagnosis by nucleic acid analysis varies directly with the multiplex scale in panel testing. Many of these applications depend on the discrimination of single-base differences at a multiplicity of sometimes close space loci.

A variety of DNA hybridization techniques are available for detecting the presence of one or more selected polynucleotide sequences in a sample containing a large number of sequence regions. In a simple method, which relies on fragment capture and labeling, a fragment containing a selected sequence is captured by hybridization to an immobilized probe. The captured fragment can be labeled by hybridization to a second probe which contains a detectable reporter moiety.

Another widely used method is Southern blotting. In this method, a mixture of DNA fragments in a sample are fractionated by gel electrophoresis, then fixed on a nitrocellulose filter. By reacting the filter with one or more labeled probes under hybridization conditions, the presence of bands containing the probe sequence can be identified. The method is especially useful for identifying fragments in a restriction-enzyme DNA digest which contain a given probe sequence and for analyzing restriction-fragment length polymorphisms ("RFLPs").

Another approach to detecting the presence of a given sequence or sequences in a polynucleotide sample involves selective amplification of the sequence(s) by polymerase chain reaction. In this method, primers complementary to opposite end portions of the selected sequence(s) are used to promote, in conjunction with thermal cycling, successive rounds of primer-initiated replication. The amplified sequence may be readily identified by a variety of techniques. This approach is particularly useful for detecting the presence of low-copy sequences in a polynucleotide-containing sample, e.g., for detecting pathogen sequences in a body-fluid sample.

More recently, methods of identifying known target sequences by probe ligation methods have been reported. In one approach, known as oligonucleotide ligation assay ("OLA"), two probes or probe elements which span a target region of interest are hybridized with the target region. Where the probe elements match (i.e. basepair with) adjacent target bases at the confronting ends of the probe elements, the two elements can be joined by ligation, e.g., by treatment with ligase. The ligated probe element is then assayed, evidencing the presence of the target sequence.

In a modification of this approach, the ligated probe elements act as a template for a pair of complementary probe elements. With continued cycles of denaturation, hybridization, and ligation in the presence of the two complementary pairs of probe elements, the target sequence is amplified geometrically, i.e., exponentially, allowing very small amounts of target sequence to be detected and/or amplified. This approach is referred to as ligase chain reaction ("LCR").

Another scheme for multiplex detection of nucleic acid sequence differences is disclosed in U.S. Patent No. 5,470,705 to Grossman et. al. where sequence-specific probes, having a detectable label and a distinctive ratio of charge/translational frictional drag, can be hybridized to a target and ligated together. This technique was used in Grossman, et. al., "High-density Multiplex Detection of Nucleic Acid Sequences: Oligonucleotide Ligation Assay and Sequence-coded Separation," Nucl. Acids Res. 22(21):4527-34 (1994) for the large scale multiplex analysis of the cystic fibrosis transmembrane regulator gene.

Jou, et. al., "Deletion Detection in Dystrophin Gene by Multiplex Gap Ligase Chain Reaction and Immunochromatographic Strip Technology," Human Mutation 5:86-93 (1995) relates to the use of a so called "gap ligase chain reaction" process to amplify simultaneously selected regions of multiple exons with the amplified products being read on an immunochromatographic strip having antibodies specific to the different haptens on the probes for each exon.

There is a growing need, e.g., in the field of genetic screening, for methods useful in detecting the presence or absence of each of a large number of sequences in a target polynucleotide. For example, as many as 400 different mutations have been associated with cystic fibrosis. In screening for genetic predisposition to this disease, it is optimal to test all of the possible different gene sequence mutations in the subject's genomic DNA, in order to make a positive identification of "cystic fibrosis". It would be ideal to test for the presence or absence of all of the possible mutation sites in a single assay. However, the prior-art methods

described above are not readily adaptable for use in detecting multiple selected sequences in a convenient, automated single-assay format.

Solid-phase hybridization assays require multiple liquid-handling steps, and some incubation and wash temperatures must be carefully controlled to keep the stringency needed for single-nucleotide mismatch discrimination. Multiplexing of this approach has proven difficult as optimal hybridization conditions vary greatly among probe sequences.

Allele-specific PCR products generally have the same size, and a given amplification tube is scored by the presence or absence of the product band in the gel lane associated with each reaction tube. This approach requires splitting the test sample among multiple reaction tubes with different primer combinations, multiplying assay cost. PCR has also discriminated alleles by attaching different fluorescent dyes to competing allelic primers in a single reaction tube, but this route to multiplex analysis is limited in scale by the relatively few dyes which can be spectrally resolved in an economical manner with existing instrumentation and dye chemistry. The incorporation of bases modified with bulky side chains can be used to differentiate allelic PCR products by their electrophoretic mobility, but this method is limited by the successful incorporation of these modified bases by polymerase, and by the ability of electrophoresis to resolve relatively large PCR products which differ in size by only one of these groups. Each PCR product is used to look for only a single mutation, making multiplexing difficult.

Ligation of allele-specific probes generally has used solid-phase capture to resolve the allelic signals, the latter method being limited in multiplex scale by the narrow size range of ligation probes. The gap ligase chain reaction process requires an additional step -- polymerase extension. The use of probes with distinctive ratios of charge/translational frictional drag technique to a more complex multiplex will either require longer electrophoresis times or the use of an alternate form of detection.

The need thus remains for a rapid single assay format to detect the presence or absence of multiple selected sequences in a polynucleotide sample.

The present invention is directed toward overcoming these deficiencies in the art.

Applicants hereby confirm the election of Group I (i.e. claims 1-88 and 138-147) in response to a telephonic restriction requirement. In addition, it is confirmed that an election of species was made which is now said to encompass claims 1-66, 75-88, and 138-147.

The rejection of claim 44 under 35 U.S.C. § 112 (1st para.), because the specification does provide descriptive support for the limitations of this claim, is respectfully traversed in view of the above amendments. Claim 44 requires that the subjecting step of claim 1 achieve "a rate of formation of mismatched ligated product sequences which is less than .005 of the rate of formation of matched ligated product sequences." In that step in claim 1, the ligated product sequence is formed from a particular one of the probe sets. Taken in this context, it is clear that the "mismatched ligated product sequences" and "matched ligated product sequences" limitations refer to one oligonucleotide probe set. By the above amendments to claim 44, it is now clear that this claim is referring to the rate of formation of mismatched litigated products versus the rate of formation of matched ligated products, where both types of products are formed from a particular oligonucleotide probe set.

The rejection of claims 12, 16, 18, 19, 20, 22, 26, 30, 34, and 81 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is respectfully traversed.

With regard to the "nearby" limitation in claims 12, 16, 18, 19, 20, 22, 26, 30, and 34, applicants submit that the meaning of this portion of the claim, when read in conjunction with the remainder of the present application, is apparent to one of ordinary skill in the art. Webster's Seventh New Collegiate Dictionary (1972) defines "nearby" as "close at hand". Moreover, Figures 5-8 exemplify this as being within up to 4 nucleotide positions apart. For all these reasons, it is submitted that the meaning of "nearby" is fully apparent to one of ordinary skill in the art. In this context, as set forth in claims 16, 26, 30, and 34, "nearby" encompasses 2 or more nucleotide base differences which would be detected by overlapping oligonucleotide probes. Thus, the ligase detection reaction procedure of the present invention is capable of distinguishing such "nearby" nucleotide differences, while other procedures like polymerase chain reaction would suffer from primer interference problems.

Claim 81 has been amended to indicate that adjacent capture oligonucleotides on the array differ "by a least 25% of the nucleotides when the oligonucleotides are aligned with one another without internal insertion or deletion." Descriptive support for this feature is found on page 40, line 14 to page 43, line 29, particularly page 42, line 17 to page 43, line 29, of the present application. In addition, for example, referring to Table 2 on page 40 of the specification, when the oligonucleotides identified as Zip11 and Zip12 are aligned end-to-end, as shown below, 9 out of 24 of the nucleotides differ. Even when the oligonucleotide

probes are not aligned end-to-end, as shown below with respect to the oligonucleotides designated Zip12 and Zip14, 11 out of 24 of the nucleotides differ.

```
Zip11      TGCG-GGTA-CAGC-ACCT-ACCT-TGCG
             || ||||       ||||    |  ||||
Zip12      ATCG-GGTA-GGTA-ACCT-TGCG-TGCG


Zip12      ATCG-GGTA-GGTA-ACCT-TGCG-TGCG
                |||| |||| ||||    |
Zip14           GGTA-GGTA-ACCT-ACCT-CAGC-TGCG
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Therefore, in view of the above amendments, the rejection of claim 81 should be withdrawn.

The objection to claims 2-5, 15, 17, 21, 23, 25, 27, 29, 31, and 33 under 37 CFR § 1.75(c) for failure to limit further the claims from which they depend is respectfully traversed. The erroneous nature of this rejection is made clear by comparison of the claims. For example, claim 1 calls for the oligonucleotide probes in a particular set to be "suitable for ligation together when hybridized adjacent to one another on a corresponding target nucleotide sequence, but have a mismatch which interferes with such ligation when hybridized to any other nucleotide sequence present in the sample". On the other hand, claim 2 calls for the probes to be "suitable for ligation together at a ligation junction when hybridized adjacent to one another on a corresponding target nucleotide sequence due to perfect complementarity at the ligation junction, but, when the oligonucleotide probes in the set are hybridized to any other nucleotide sequence present in the sample, have a mismatch at the base at the ligation junction which interferes with such ligation." Meanwhile, claim 4 requires that the probes be "suitable for ligation together at a ligation junction when hybridized adjacent to one another on a corresponding target nucleotide sequence due to perfect complementarity at the ligation junction, but, when the oligonucleotide probes in the set are hybridized to any other nucleotide sequence present in the sample, there is a mismatch at the base adjacent to the base at the ligation junction which interferes with such ligation." Claims 15, 17, 21, 23, 25, 27, 29, and 31 similarly add limitations like those of claim 2, while claim 33 has limitations like those of both claims 2 and 4. Since the objected to claims

further limit where a ligation precluding mismatch can be located, they are proper dependent claims and the objection should be withdrawn.

The rejection of claims 1-5, 11-21, 24-43, 45-66, 75-77, 79-80, 83, 87, 88, and 138-147 under 35 U.S.C. § 103 for obviousness over Wiedmann, et. al., "Ligase Chain Reaction (LCR)—Overview and Applications," PCR Methods and Applications pp. S51-S64 (1994) ("Wiedmann") in view of Barany, "The Ligase Chain Reaction in a PCR World," PCR Methods and Applications pp. 5-16 (1991)("Barany PCR"), U.S. Patent No. 5,415,839 to Zaun et. al. ("Zaun"), and Guo, et. al, "Direct Fluorescence Analysis of Genetic Polymorphisms by Hybridization with Oligonucleotide Arrays on Glass Supports," Nucl. Acids Res. 22(24): 5456-65 (1994)("Guo") is respectfully traversed.

Wiedmann describes the use of the ligase chain reaction ("LCR") procedure to detect single base differences in target nucleic acids. LCR is disclosed to involve the use of 2 sets of oligonucleotide probes. The first set of probes is configured to hybridize to a first nucleic acid strand, while the second set of probes is designed to hybridize to a second nucleic acid strand complementary to the first nucleic acid strand. The first and second sets of oligonucleotide probes are complementary to one another. As a result of using both sets of oligonucleotide probes targeted to complementary nucleic acid strands, the LCR procedure is able to achieve exponential amplification. Wiedmann distinguishes the LCR procedure from the ligase detection reaction ("LDR") technique by virtue of the fact that LDR only uses a single set of oligonucleotide probes which will hybridize to only one target nucleic acid strand and, thus, achieve linear amplification. There is no suggestion in this reference of using LDR in conjunction with a solid support to capture ligated product sequences resulting from LDR by hybridization to a capture oligonucleotide.

Barany PCR is substantially the same as Wiedmann with the former being relied upon in the outstanding office action as teaching signal to noise ratios, various reporter groups, multiplex formats, detection of multiple mutations, operating conditions, and the use of thermostable ligases. Like Wiedmann, Barany PCR does not suggest using LDR in conjunction with a solid support to capture ligated product sequences resulting from LDR by hybridization to a capture oligonucleotide.

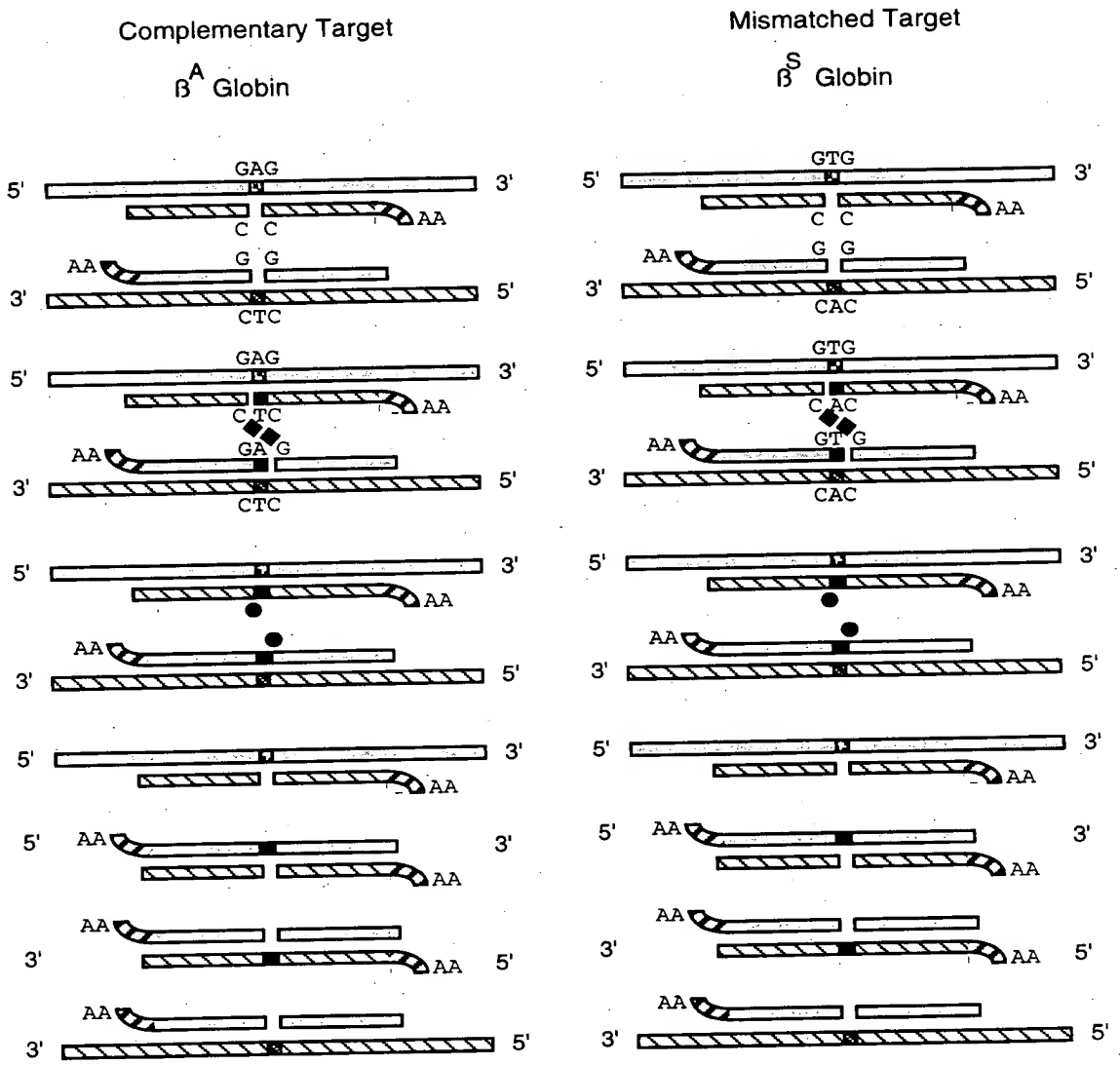
Zaun discloses an apparatus and method for amplifying and detecting target nucleic acids. This procedure involves amplifying with a thermal cycling device and then detecting reaction products on a support having one or more reaction sites. Amplification can be carried out using the polymerase chain reaction ("PCR") or LCR procedures. To capture

amplification products, the detection system is provided with a support having a plurality of capture sites to immobilize such products on the support. Zaun discloses capturing amplification products with antibody-antigen binding, chemical bonding, or complemention of polynucleotides.

As noted *supra,* Zaun, at most, relates to use of LCR as an amplification procedure which is distinguishable from LDR. Moreover, although Zaun refers to a number of LCR procedures (see col. 1, lines 50-62), the LCR procedure being practiced in that reference (see col. 39, lines 1 to 11) is very different from that described in Barany. More particularly, the LCR procedure utilized by Zaun references EP-A-439,182 to Backman, et al., (copy enclosed) which is known as gap-LCR. The difference between gap-LCR and the LCR procedure of Barany is fully explained in Wiedmann (compare Figures 1 and 4). In brief, Barany's LCR involves use of oligonucleotide probe sets which hybridize to a target nucleic acid in abutting relationship, and, if there is perfect complementarity at their junction, these oligonucleotides can be joined with ligase. By contrast, in gap-LCR, the oligonucleotide probe sets do not hybridize to a target nucleic acid in abutting relation and, therefore, are not potentially suitable for immediate ligation. Instead, there is a gap which must be closed using polymerase before any ligation can occur.

This difference between Barany's LCR and gap-LCR is more than a mere difference in methodology. This difference has a significant impact on the utility of each technique. For example, in detecting sickle cell anemia, the distinction between the normal $ß^A$ and sickle $ß^S$ genes is a single A → T transversion which leads to a change from a glutamic acid residue to a valine in the hemoglobin ß chain. This single base difference is readily detected with the LCR procedure of Barany. See also Barany, "Genetic Disease Detection and DNA Amplification Using Cloned Thermostable Ligase," Proc. Nat'l Acad. Sci. USA 88:129-93 (1991) ("Barany PNAs"). Gap-LCR is able to distinguish the single base difference between *L. monocytogenes* and *L. innocua.* See Figure 4 of Wiedmann. However, when this procedure is applied to detection of sickle cell anemia, it is ineffective as shown below, because it forms the same ligation product when the $ß^A$ globin gene and $ß^S$ gene are present.

1. Denature DNA, 94°C. Anneal oligonucleotides, 65°C.
2. Fill in with only dATP and TTP using *Taq* polymerase.
3. Ligate with thermostable ligase at 65°C.
4. Repeat cycle 30 to 60 times.



Complementary Target cannot be distinguished from mismatched Target using a Gap-LCR reaction.

In distinguishing, *L. monocytogenes* from *L. innocua*, the single base difference involves A in the former and G in the latter, which are not complementary. When dATP and TTP are added to the sample, the missing A base (and its complementary T base) are filled for the *L. monocytogenes* matched target but not for the mismatched *L. innocua* target. Thus, where the base differences between matched and mismatched targets are not complementary, gap-LCR can be used to detect single base differences. However, when the bases distinguishing matched and mismatched target are complementary (e.g., A for the $ß^A$ globin gene and T for the $ß^S$ globin gene), gap-LCR is ineffective.

The claims of the present application clearly refer to an LDR procedure and require that the oligonucleotide probes be configured to hybridize "adjacent to one another" on a corresponding target nucleotide sequence. Since they do not involve LCR or filling a gap, these claims are readily distinguishable from Zaun.

Moreover, Zaun completely fails to provide an enabling disclosure of a hybridization capture procedure. The bulk of that disclosure relates to the use of hapten-antibody binding to effect capture. There is nothing to suggest that the particular technique of wicking reaction product on a strip to a capture site is useful for any mode of capture other than that dependent on hapten-antibody binding. Although there is brief mention of utilizing complementary polynucleotides, there is little detail about how this is to be carried out in conjunction with the overall process of Zaun. To the extent Zaun is referring to a hybridization mode of capture, there is no reason to think that Zaun is hybridizing to anything other than to the target specific portion of the ligation product from the LCR procedure. By contrast, the claims of the present invention call for a pair of oligonucleotide probes where one has an "addressable array-specific portion" which is complementary to a capture oligonucleotide on a solid support. By utilizing a probe set where one probe has an addressable array-specific portion which is separate and distinct from a target specific portion, the capture phase for the process of the present invention can be carried out with different oligonucleotide probe sets having similar melting temperatures and hybridization kinetics. As a result, multiplex processing and quantitative measurement is facilitated. By contrast, when the portion of the probe both binds to the target nucleic acids in a sample and capture probes on a support, the different probe sets will require different melting temperatures and hybridization kinetics.

Thus, Zaun in no way suggests the present invention.

Guo relates to a multiplex PCR amplification procedure. This involves amplifying and detecting target nucleic acids using fluorescently labeled tags where the amplification products are denatured, captured on a solid support with oligonucleotide probes, and detected. Like Wiedmann, Barany, and Zaun, Guo does not suggest using LDR in conjunction with a solid support to capture ligated product sequences resulting from LDR by hybridization to a capture oligonucleotide. In addition, as discussed above with respect to Zaun, the solid support of Guo employs capture probes like those of the target material to hybridize to a target-specific portion of a PCR extension product. This is very different than using an oligonucleotide probe with an addressable array-specific portion for capture which is separate and distinct from a target-specific portion. Thus, Guo fails to suggest the present invention.

Neither Wiedmann, Barany PCR, Zaun, nor Guo suggest using an LDR procedure with oligonucleotide probe sets where one probe has an addressable array-specific portion separate and distinct from a target-specific portion. The combination of these references also fails to suggest a detection procedure involving capture of the resulting ligated product sequences by hybridization of the separate and distinct addressable array-specific portion to a capture oligonucleotide. Accordingly, the rejection based upon this combination of references should be withdrawn.

It is further submitted that this combination of references also fails to teach the parenthetical features of the following dependent claims: 4 (mismatch at a base adjacent to a base at the ligation junction), 11 (quantifying target nucleotide sequences), 12, and 14-34 (detecting multiple allele differences), and 13 (target specific portions of oligonucleotide probes with substantially the same melting temperatures). Therefore, even if the rejection based on the combination of Wiedmann, Barany PCR, Zaun, and Guo is not withdrawn with respect to all the independent claims, at least claims 4 and 11-34 should be allowed.

The rejection of claims 6-10, 22, and 23 under 35 U.S.C. § 103 for obviousness over Wiedmann in view of Barany PCR, Zaun, Guo, and Telenti, et. al., "Competitive Polymerase Chain Reaction Using an Internal Standard: Application to the Quantitation of Viral DNA," J. Virol. Methods 39: 259-68 (1992)("Telenti") is respectfully traversed.

Telenti is cited as teaching that PCR can be quantitated by providing a known amount of an internal standard. However, this reference does not disclose the use of an internal standard in conjunction with an LDR process nor does it involve the use of arrays.

PCR is an exponential amplification procedure, while LDR is a linear amplification technique. In addition, competitive PCR internal standards work where primers are all identical for the unknown target and the known internal standard. By contrast, for LDR, the discriminating probes for the unknown target and the internal standard must differ. Therefore, teachings regarding the internal standards for Telenti's PCR procedure are not readily adaptable to the LDR procedure of the present invention. Since Telenti does not overcome the above-noted deficiencies of Wiedmann, Barany PCR, Zaun, and Guo, the rejection based on this combination of references must be withdrawn.

The rejection of claim 44 under 35 U.S.C. § 103 for obviousness over Wiedmann in view of Barany PCR, Zaun, Guo, and Barany PNAS is respectfully traversed.

Barany PNAS is cited for its teaching of LDR mismatched:matched ratios. However, this reference is substantially similar to Wiedmann and Barany PCR and, therefore, cannot overcome the above-noted deficiencies of Wiedmann, Barany PCR, Zaun, or Guo. Therefore, the rejection of claim 44 should be withdrawn.

The rejection of claims 78, 82, and 84-86 under 35 U.S.C. § 103 for obviousness over Wiedmann in view of Barany PCR, Zaun, Guo, and Sambrook, et. al., Molecular Cloning (1989)("Sambrook") is respectfully traversed.

Sambrook is cited for its teachings regarding hybridizing to immobilized nucleotides, barrier oligonucleotides, exonucleases to destroy nucleotides, and stripping blots. Although Sambrook may provide such general teachings, it in no way suggests using these techniques in conjunction with the process of the present invention which combines LDR with capture of ligation products by hybridization to a solid support. In view of these deficiencies, the rejection based on the combination of Wiedmann, Barany PCR, Zaun, Guo, and Sambrook should be withdrawn.

With regard to the outstanding office action, the PTO-1449 forms accompanying the Information Disclosure Statement filed simultaneously with the present application were not initialed and returned to applicants. It is respectfully requested that this be done with the next written communication from the U.S. Patent and Trademark Office. For convenience, copies of the PTO-1449 forms are enclosed.

In view of all the foregoing, it is submitted that the present application is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date June 13, 1998

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